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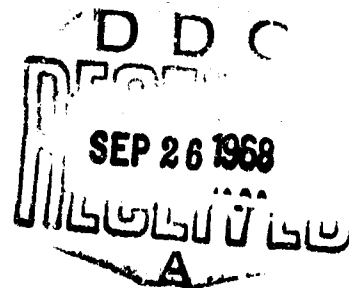SMUFD D/A ltr, 17 Feb 1972

AD840032

TRANSLATION NO. *3572*

DATE *July 1968*

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SEP 26 1968

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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

ELECTRON OPTICAL INVESTIGATIONS ON PARAVACCINIA VIRUS (V. PIRQUET)

(Following is the translation of an article by T. Nasemann
and E. Bauer, Dermatology Clinic and Physical Institute of
the University of Munich, published in the German language
periodical Klin. Wochen. 35, pages 62-67 (1957). Translation
performed by Constance L. Lust.)

In 1892 Danve and Larue reported concerning an anomolous reaction
during vaccination against pox. They designated it according to the
morphologic picture as "Vaccine rouge" (cherry red pimples in the area
of the vaccination.) The viruses isolated from these red pimples be-
haved biologically different than Variola vaccine virus. Because of
this v. Pirquet designated the agents of the "red vaccination reaction"
as Paravaccinia. In 1919 Lipschuetz (1919, 1923) gave the elementary
bodies of paravaccinia the name Strongyloplasma paravaccine.

Studies by Nasemann and Deubner (1953) and Marchionini and Nasemann
(1955) indicated that the "Vaccine rouge" and the actual Melkerknoten
(sensu strictiori in the sense of Berger 1955) are caused by the same
paravaccinia virus. Clinical and experimental details about the identity
of the two illnesses and the etiology of the disease, can be found in
the above references as well as those of Kaiser (1952), Katzenellenbogen
(1952), Sonck and Penttinen (1954), Bosse (1956), and Nasemann (1956).

Clinical Observation

Jenner at the end of the 18th century already differentiated between
the real (actual) Cowpox and the so-called "Fake pox" (Euteropox). The
demarcation of the original Cowpox from Variola vera and the present
vaccinia virus derives from the studies of Downies (1939,50,53). False
pox on the udder of cows are the source of infection for paravaccinia.
Cows infected with euteropox as a rule show no general symptoms; the
animals have no pain during milking, milk production is normal and appetite
remains healthy. Contamination of stables with euteropox may lead to
recurrent illnesses for weeks or months; animals may be ill several times.
After recovering from the infection only minimal (or none) immunity re-
mains. During milking the paravaccinia virus enters the host via epithelial
defects of the skin and leads to specific changes of the epidermis and
Corium (hyperkeratosis, degeneration of Rate-cells, eosinophilic intranuclear
and intracytoplasmic inclusion bodies, lymph and leucocytotic infiltration
of differing degree). Histological studies showed some change in the
granuloma teleangiectaticum. Clinicially and histologically these cor-
responds well to each other for both diseases. Complaints arise only
when a secondary bacterial occurrs. The nodes due to paravaccinia dis-
appear in several weeks. The appearance of vaccine rouge after vaccination
may be explained by using calf lymph which had been unintentionally
contaminated by material of euteropox. (This is rare in Germany; was

1.

very common in Balkan countries). In patients with nodes due to para-
vaccinia a protective immunization with variola vaccine is positive
(takes). Sonck and Pentitinen (1954) reportedly transferred the para-
vaccinia germ from human to human, despite the presence of vaccine im-
munity. Berger (1955) later successfully revaccinated previously vac-
cinated cows.

Biological Characteristics of the Paravaccinia

In stained streak-preparations (Victoria blue, Carbolfuchsion) the
elementary-bodies of paravaccinia cannot be differentiated from those of
variola vaccinia. Lipschuetz already pointed this out. However, bio-
logically there are clear differences. Contrary to vaccinia virus, para-
vaccinia cannot be transferred to rabbit cornea nor on to the chorioallan-
toic membrane of incubated chick eggs. This varying behavior lead to a
separation of terminology from the morphologically similar variola vaccinia.
The present electron-optic studies were designed to test how far (to what
extent) the paravaccinia and variola vaccinia agree in their micromorphology.

Methods and Materials

We have examined fresh, excized material from eight paravaccine in-
fected nodes of udder with virologic methods. During these studies we
could regularly demonstrate the virus elementary body (ELK) light optically
in streak preparations (also see Nasemann and Deubner 1953). We could
not transfer virus dispite high virus content onto corneas or eggs. After
vaccinating skin (persinal test) of the upper thigh a cherry-red pimple
appeared (similar to vaccine rouge). We only examined two nodes histo-
logically. The above mentioned alterations were found. In serum of
patients no vaccinia antibodies (KBR) were found. Since we could find
no host cell to grow the virus (except from bovine) eg. egg, mouse cells,
rabbit cell line, we could produce no specific paravaccinia antigen.
An infected herd of cows near Munich could as yet not be determined.
For these reasons we could use only the infected excized tissue (in-
fected human skin) for the electron microscopic studies. Perhaps this
lack can be alleviated with aid of tissue culture studies. Such studies
are now in preparation.

The nodes of three patients were studied with the electron microscope.
Small tissue particles were either pressed carefully onto the object
carrier (Zapan treated platinum net) or were indirectly picked up on glass
object carriers before drying. In this way the preparations are not very
clean since tissue constituents are also picked up. Dispite this we ob-
tained numerous pictures of paravaccinia virus which we could evaluate.
We used an AEG Zeiss electronmicroscope. A portion of the preparation
was treated obliquely with palladium.

The enzymatic experiments were done according to Peters and coworkers.
(Peters and Nasemann 1952,53, Peters and Stoeckenius (54 a,b,c), Stoeckenius
and Peters (1955), Nasemann and Huber 1955). For fixation Chabaud-mixture
was used 15 min. A part was treated with pepsin (0.02% solution of cry-
stelline Worthington Pepsin, pH 2.0, 37°C 2 hrs; another part was subjected

2.

to stepwise degradation with pepsin and DNAase (pepsin 2 hrs., then
0.1% solution of crystalline DNAase pH 6.0, 37°C, 30 min; pepsin
2 hrs). Every time the solution was changed the preparation was washed
two times with new distilled water.

The quantitation of length and width of paravaccinia ELK was done
on the "clearest" pictures of the "clearest" preparation. We could
only measure 20 ELK clearly. The size determination should only be
used as a preliminary result. This result await growing this virus in
passages in cultures.

Results

Like the (ELK) elementary particle of variola the paravaccinia
possess parallelepiped (Ashler) form. They are prima vista not distin-
guishable. Paravaccinia also belongs morphologically in the pox group.
Length 296 mu (range 165-360 mu), width 190 (165-230). The elementary
particles (ELK) lie unregularly distributed between detritus masses
(see figure 1). This cell material can be largely illiminated by treat-
ment with pepsin (figure 5). Occasionally intact skin lamellae were in
the preparations. ELK was found between them also. This may be observed
in figure 2. In figure 3 the parrallelepipet form of ELK is well recorded.
The corners of the form are rounded off. This may be seen even better
in figure 4.

Similar to the other viruses with this structure (Variola etc) para-
vaccinia may be partially degraded with pepsin. This results in mem-
brane-like forms, which surround a central core (not attached) of variable
size; also completely empty membranes and a few intact ELK (see figure
5).

If the enzymetic degradation occurs stepwise, pepsin 2 hr. - DNAase
30 min. - pepsin 2 hr., then the central body is also dissolved. Then
only empty membranes are found inside which may be found very small, dense
bodies (see figure 6). No difference in behaviour to pepsin and DNAase
existed between the variola and paravaccinia.

Discussion

Paravaccinia virus is a little more slender than variola and some-
what smaller than Molluscum contagiosum virus. Length ELK of vaccinia
240-380 mu, width 170-270 (Peters and Nasemann 1952a). Median length of
M. contagiosum virus 316 mu - 17%, width 245 - 18% (Peters 1954a).
Pepsin attacks paravaccinia ELK as it typically does pox viruses. By
treating pepsin and DNAase in combination the DNA containing core is also
degraded. The micromorphology of paravacinnia and the other pox viruses
is very similar in all ways. They vary clinically and microbiologically.
One causes epidermal lesions and cannot be transferred to lab animals or
eggs. Paravaccinia causes changes in the epidermis as well as in the
Corium; it infects bovines and humans. Humans and a number of animals
are susceptible to variola vera and vaccinia virus. Skin and other

tissues are affected. Human and animal pox viruses may be grown in egg cultures. In table 1 the difference between variola - and paravaccinia are summarized. It should be noted that paravaccinia virus is only the pathogen for disease in the sense that Berger (1955) described and is not itself identical to the alterations of cowpox or of the so called vaccinia knots. If vaccinia or cowpox cause an infection, egg culture of excized material is positive and in the patients serum a rise of antibody is found.

Summary

the virus diameter from

Paravaccinia virus (v. Pirquet) belongs to pox type. By electron microscopy the parallelepiped form was demonstrated; length 296 mµ and width 190 mµ. Pepsin and DNAase attack ELA in the same way as the other pox viruses. Microbiologically there are some differences between variola and paravaccinia virus (table 1).
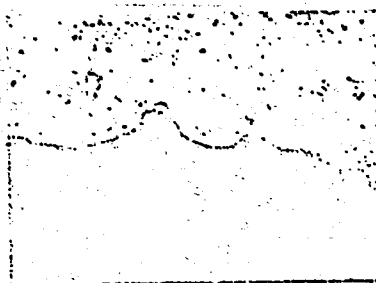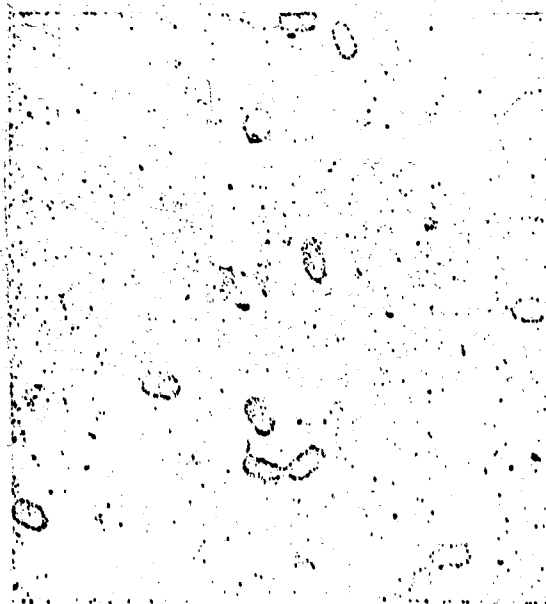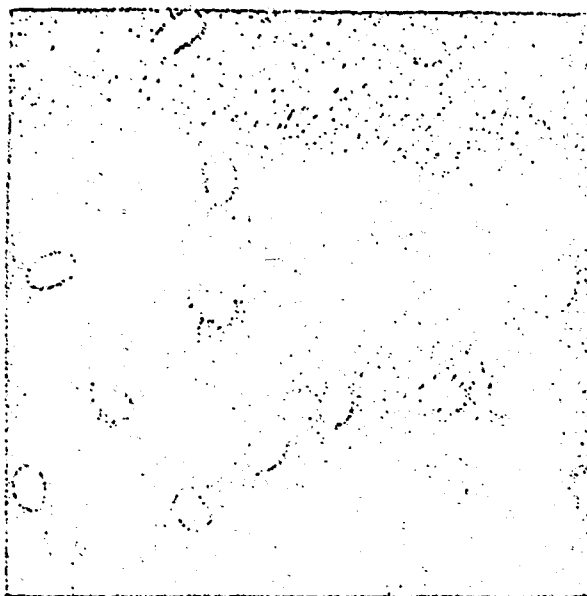
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Figure 1

Figure 2

Figure 3

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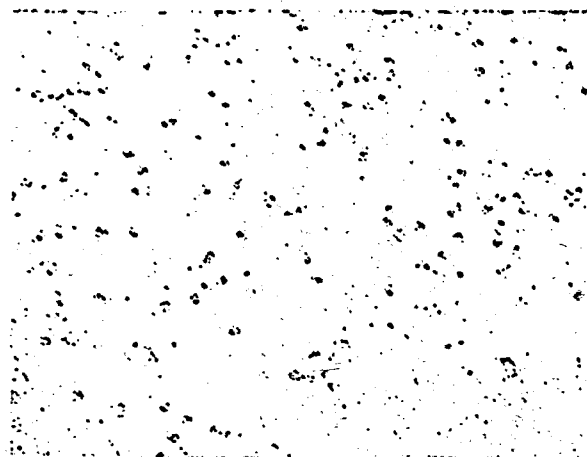
Figure 4

Figure 5

Figure 6

Table 1 - Differences between Variola and paravaccinia

	Variola	Paravaccinia
Paulscher comm. trial	+	−
egg culture	+	−
human to human transfer	+	+
C.F.+	+	−
light optic ELK	+	+
Electron optic	parallelepipet of ELD	same
histology	intracytoplasmic	intranuclear and intracytoplamic

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